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An Exploration of Possibly Using Anti-Angiogenic Therapy as a Treatment for Lung Cancer

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Abstract

Controlling angiogenesis *in vivo* is strongly being considered by pathologists and experimenters due to the recent success of using anti-angiogenic therapy for cancer patients in clinical trials. The apparent nature and overwhelming number of cases of lung cancer (bronchogenic carcinoma) are leading some clinicians to using anti-angiogenic pharmaceuticals with lung-cancer patients. Pre-clinical and experimental evidence supporting the use of anti-angiogenic therapy for lung cancer is apparently increasing. The literature research here attempts to point out specific relationships between lung tumor growth and the mechanisms by which the body reacts to such. Macrophages, lymphocytes, fibroblasts, vascular smooth muscle cells, lung alveolar, and vascular endothelial cells all play roles in such wound-healing mechanisms as inflammation, angiogenesis, and fibrogenesis that involve growth factors and that are activated in the presence of an invasive lung neoplasm. Elaboration on many of these cell-extracellular matrix interactions of normal and tumorous tissues might lead to a better understanding and a stronger foundation for being able to utilize or derive a highly accurate anti-angiogenic therapy for one of the largest causes of cancer-related mortality.

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Introduction to Anti-angiogenesis

"If anti-angiogenesis is not possible, or even if the concept is wrong, the careful exploration of its consequences may reveal something fundamental about the behavior of tumor cells growing in a packed population *in vivo* (1)." Though the exploration and research of anti-angiogenic therapy continue to accelerate, clinicians and researchers have yet to uncover a sufficient explanation for tumor behavior that would lead to a cure for cancer. Still, this persistent endeavor to inhibit the intricate process of blood vessel formation, or angiogenesis, certainly reveals something fundamental about it with respect to tumor growth.

Angiogenesis is a developmental process whereby new blood vessels form from existing vasculature and grow into a relatively unvascularized tissue matrix. Vascular endothelial cell proliferation and migration are requisite steps in angiogenesis and are probably the most important in the identification of its presence (2). More specifically, proliferating vascular endothelial cells' dependency on specific interactions with extracellular matrix (ECM) constituents of their basal lamina is essential in the angiogenic role of activated-macrophage growth factors(2,3). Growth factors, ECM components, and various inflammatory cytokines (cell-derived activators) are all utilized

by specific types of blood and connective tissue cells to mediate angiogenesis, inflammation, and ultimately wound repair (2-6).

Angiogenesis is a definitive characteristic of any angioproliferative neoplasm, i.e. new, abnormal growth (e.g. a solid tumor), which is replete with investing vasculature. The phenotypic nature and metabolic requirements of the tumorous tissue are supposedly what determine the degree of angiogenic activity the tumor induces from the surrounding vascularized tissue (6).

Still, all tumorous growths are not so heavily dependent on angiogenesis as a central pathogenic step. Early on, it was pointed out that neither high microvessel density nor counts of angiogenic activity are characteristic of all solid tumors. In Folkman's original hypothesis, he noted that sarcomas (cancers of connective tissue), especially chondrosarcomas, do not have highly active vascular endothelial cells or profuse capillary beds as do gliomas (non-nerve cell tumors in the nervous system) and carcinomas (cancer of epithelial tissue origin) but are still able to form relatively large neoplasms (1). Therefore, angioproliferative diseases such as lung carcinomas, prostate cancer, breast cancer, gliomas, and some sarcomas (e.g. Kaposi's sarcoma) are the targets of much of the present anti-angiogenic clinical trials and experimental research on angiogenesis (7-14).

The idea that solid tumors might become dormant or regress by therapeutically inhibiting angiogenesis was formally introduced by Dr. Judah Folkman in 1971(1). Supposedly inhibiting angiogenesis would reduce an angiogenic tumor's vital supply of nutrients and cause the tumor to regress or at least become dormant (15,16). To better understand angiogenesis in tumors, new angiogenesis assays as well as the technology to

distinguish between what proteins or factors were angiogenic, anti-angiogenic, or simply nonangiogenic had to be developed (1,12,16-19). Since that time, a wealth of research has been devoted to the study of biochemicals and mechanisms relating to angiogenesis, almost solely to find a novel cancer therapy less toxic and more efficient than surgery, radiotherapy, or chemotherapy (8,11,13,20-22).

Anti-angiogenic Therapy's Applicability to Bronchogenic Carcinoma

During the past 27 years, intense investigations of angiogenesis have led to the discovery of many angiogenic proteins, inhibitory and stimulatory. For example, endostatin, a human collagen XVIII fragment, causes significant regression in tumors *in vivo* without inducing the drug resistance commonly following application of any cytotoxic chemotherapeutic drug (23). Therapeutic agents such as suramin, retinoic acid, and heparin-steroid combinations that were originally used with success as pharmacological agents, have all been shown to have angiostatic or anti-angiogenic activity (24-30). However, depending on the extent of the cancer's progression, the effective dosage required of these broadly applicable agents might lead to uncertain side effects other than cytotoxicity that occurs from current chemotherapeutic agents (31) and combinations.

Recently, responses to the results of experimental angiogenic studies have led to the initial clinical trials of anti-angiogenic agents such as retinyl palmitate, 13-cis retinoic acid, marimastat (matrix metalloproteinase inhibitor), TNP-470, carboxyamidotriazole, and tecogalan sodium (7,8). The retinoids and marimastat are currently going through clinical trials involving non-small cell lung cancer (NSCLC: a type of bronchogenic carcinoma) patients and, thus far, indicate a significant reduction of secondary tumors

and further metastatic activity. These are the expected effects aside from hopes of reducing the size of tumors (7).

Anti-angiogenic therapy is, at least, expected to halt the growth of blood vessels. The removal of a therapy based on a cell-mediated pathway may allow the tumor mass to repeat its development and induction of angiogenesis with a relatively larger mass of cells and blood vasculature (22,23). Without further anti-tumoral therapy or modifications, current anti-angiogenic therapies can certainly not be considered the cure for cancer.

The anti-angiogenic therapies of these initial clinical trials are adjuvant therapies, or post-therapeutic therapies, for patients having already had surgical resection of a tumor or who have responded to chemotherapy. Though the angioproliferative tumors theoretically fall dormant after their angiogenic activity is halted, they are not inhibited from remaining viable *in vivo* with respect to the extent of their established vasculature (7,11,15). In tissue culture, tumor cells generally proliferate regardless of the previous number of cell divisions or attachment and orientation to other cells, veritably comparable to the extent that a bacterial colony proliferates (32). These tumor cells are usually not recognized by the immune system except as an invasive mass; thus, angiogenesis and inflammation may continue to increase, regardless.

Though anti-angiogenic therapy is considered a promising adjuvant therapy, major questions concerning the state of the resulting dormant or suppressed tumor remain to be answered completely. Anti-angiogenic therapy has yet to undergo extensive clinical trials and should not be considered an anti-tumoral therapy but rather an anti-proliferative or anti-metastatic adjuvant to anti-tumoral combination therapies of surgery,

radiation, and chemotherapy (7,9,10). To date, combination therapies of surgery, radiotherapy, and chemotherapy are the most successful anticancer therapies for the most extreme cases of bronchogenic carcinoma (non-small cell and small cell lung cancer), one of the leading causes of mortality as a result of cancer (20,21,31,33).

In the less severe stages of lung cancer, complete recovery is often achieved using surgery or multiple-agent chemotherapy alone (7,33-35). Hopes that the same might be true for anti-angiogenesis are slim. The use of anti-angiogenesis as an adjuvant to tumor-specific cytotoxic therapy is considered much more feasible at this stage in clinical trials.

However, one of the most significant findings that anti-angiogenesis could help remedy is the correlation between the incidence of metastasis and the degree of angiogenesis within and around lung neoplasms. Studies of anti-angiogenic therapy and lung neoplasm development have been successful by revealing new agents to halt metastasis (7,33). According to the Textbook of Pulmonary Diseases, the lungs are the most frequent site of metastasis from nearly all organs: cancers of the colon, kidney, breast, testis, uterus, head and neck, ovary, as well as sarcomas and melanomas all potentially metastasize to the lungs (33).

Metastasis requires the invasion of the blood-vascular system by the tumor cells and the potency of those cells to reproduce and to become lodged elsewhere in the body after having bypassed the immune defenses. Blood filtering through the lungs allows tumor cells to become lodged in the lungs' capillary bed (33). If not cleaned up by the filtering mechanisms of the liver and lymph system or phagocytic cells of the immune system, then the embolic tumor cells may proliferate, thereby forming secondary tumors. Lymph nodes are one of the first places, besides the lung, that bronchogenic carcinomas

metastasize to, thus resulting in node-positive rather than node-negative lung cancer. N-stage (N-0,1) is a clinical measure of whether or not the lymph nodes that are closely associated to the lungs have been metastasized to and a determinant of the stage to which the cancer has progressed (stages I,II,IIIa,b,IV or T-0,1,2,3,4; stage IV being a highly metastatic, node-positive, fully-progressed, invasive bronchogenic carcinoma). Lymph-node metastasis is one of the best clinical determinants of whether a cancer is metastatic or not.

It is important to consider the necessity of using anti-angiogenic therapy for lung cancer with respect to the type of lung cancer and its prognosis. Often, surgical resection alone of operable lung carcinomas is enough to sterilize any residual cancer (33,36). Even in some stage III lung cancer cases, both chemotherapy and surgery can completely sterilize the cancer in extremely favorable conditions. Small cell lung cancer (SCLC) is extremely aggressive and invasive early in its development, thus very susceptible to certain chemotherapeutic or chemotherapy/radiotherapy treatments (17,33). After most surgical resections of the lung tumor mass in patients with stage I non-small cell lung cancer (NSCLC), the recurrence rate is about 25% to 40%, indicating that the original tumor mass was metastatic to a degree that predominated the immune response in some patients (36). Though NSCLCs (squamous cell, adenocarcinoma, and large cell) are slow growing, they are still invasive and may pose a greater threat than SCLCs in terms of metastatic ability and persistence. One study comparing types of NSCLCs even demonstrated a significantly greater correlation of angiogenesis to metastasis with adenocarcinoma than with squamous cell carcinoma (37).

As stated before, however, many of the results from the use of combination therapies of surgery and chemotherapy are not significantly increasing the survival of the patients. Using anti-angiogenesis in many experimental studies, and especially in the few clinical trials with NSCLC patients conducted thus far, is found to significantly prevent metastasis or the recurrence of secondary neoplasms. In a clinical study of 87 patients with stage I node-negative NSCLC, metastases developed in 22 patients after having radical surgical resections; respectively, microvessel count and density grades are the only significant predictors of metastasis (34). If anti-angiogenic therapy ultimately gives rise to an adjuvant, anti-metastatic therapy, physicians may be able to eliminate two negative prognostic factors instead of only one.

Results from numerous clinical studies with lung-cancer patients have practically solidified the correlation between metastasis and angiogenic activities. According to a clinical study of 28 patients who had already undergone chemotherapy and/or radiation treatment, and surgical resection for stage IIIb NSCLC, peritumoral blood and lymph vessel invasion by tumor emboli is highly significant to the incidence of metastasis and to tumors which induced angiogenesis (38). Furthermore, a study of 253 NSCLC patients clarified that microvessel count is significantly related to hilar or mediastinal node metastasis according to a univariate analysis ($P<0.000001$), a logistic regression analysis ($P<0.000003$), an overall survival analysis (log-rank test $P=0.00067$, Kaplan-Meier method with 94 patients showing metastatic disease) (39). Later on, another study of 107 operable (T1,2–N0,1) NSCLC carcinomas confirmed that vascular grade is significantly correlated to the incidence of lymph-node metastasis. The same study also compared vascular grade to other prognostic factors (histology, N-stage or nodal involvement,

epidermal growth factor receptor, proliferation index, and p53 expression), confirming that both N-stage and vascular grade are very significantly related to poor prognosis for survival and that angiogenesis (vascular grade) is the only factor significantly related to nodal metastasis (40). The possibility that angiogenesis is a strong negative prognostic factor is also confirmed by a study of 275 consecutive NSCLC patients (36). Several other studies and reviews show that both indirect and direct correlates to angiogenesis are also strongly correlated to poor prognosis as well as to lung tumors' metastatic ability (9,33,41-44).

Even though angiogenesis is strongly related to metastasis and inflammatory and angioproliferative lung diseases, the use of anti-angiogenesis as a preventive measure might inhibit immune reactivity towards recognized antigens. The ability of a lung tumor to metastasize to a lymph node certainly indicates that the immune system may not be potent enough to suppress a tumor's proliferation. Inhibiting angiogenesis could prevent the tumor from metastasizing, but it could also reduce the effectiveness of other anti-tumoral therapies that are dependent on intravenous action. Some of the more recent immunotherapeutic techniques are able to enhance the cytotoxic (anti-tumoral) activities of lymphocytes but have generally produced results similar or less satisfying than the combination therapies of surgery, radiation, and chemotherapy for extreme cases and have resulted in no significant increase in overall survival (7,20,45). If these particular immunotherapeutic techniques were to be used in combination with anti-angiogenesis, the resulting combination therapy might have a less potent overall anti-tumoral effect.

Beyond anti-angiogenic therapy currently being an adjuvant to anti-tumoral therapies, the number and extent of its consequences must be further evaluated

experimentally as well as clinically. Angiogenesis also occurs naturally in the human body under specific circumstances and does not strictly pertain to tumor growth. Inhibiting angiogenesis might interfere with various wound-repair mechanisms or placental development. Anti-angiogenesis has a broad range of effectiveness and, even when developed therapeutically, could still be met by strict regulation via natural body mechanisms, especially if the body has not already recognized the impinging nature of the tumorous growth.

Clinical and Experimental Evidence Concerning the Use of Anti-angiogenesis for Bronchogenic Carcinoma

Angiogenesis, Wound Repair, and The Extracellular Matrix

Research in angiogenic mechanisms has come close to paralleling the research on inflammation, wound-healing mechanisms, and tumors' invasiveness into existing stromal matrices, basal laminae, and basement membranes. Currently, researchers continue to acknowledge the importance of extracellular-matrix molecules' role in tumoral angiogenesis. Fibrin, thrombin, plasmin, laminin, type IV collagen, heparan sulfate proteoglycan, and integrins all play a role in stabilizing the interstitial matrix, basal lamina, and basement membrane under normal conditions; each of these proteins or protein groups is recognized experimentally as also having a role in the mechanisms by which capillary outgrowths proliferate.

In 1974, Gimbrone et al. developed one of the first *in vivo* angiogenic assays, the rabbit corneal micropocket assay, in response partly to the increasing awareness of cancer and partly to studies on silver cauterization (using silver nitrate as a local anaesthetic and cauterizing the tissue to create a sterile wound of the cornea) (12,29,30). In their original assay, a small micropocket was created in the rabbit cornea – a transparent, avascular tissue with a rigid connective tissue – via a probe, and a small sample of a tumor (a gel for control) was inserted into it. The results simply came from visual examination of the

cornea and of tissue slides created from various stages in the growth of the tumor sample and of the microvessels that protruded from the blood vessels surrounding the cornea (limbal vessels). Gimbrone et al. found that even control samples in the micropockets could develop an angiogenic response, though nowhere near the degree that the tumor samples did. This could partly be expected when one considers that the wounding of the cornea during the micropocket formation or cauterization may also elicit inflammation and edema within the corneal tissue. Regardless, the fact that angioproliferative tumors consistently experienced exponential growth after microvessel invasion of the tumor stroma suggests that angiogenesis plays an extremely important role in tumor growth.

Even before the early 1970's, the angiogenic response was associated with wound healing – whether the wounds are the result of a tissue disorder or a surgical procedure (12,46-49). The disruption of the extracellular matrix and epithelial basement membrane of the cornea by cauterization releases the extracellular and cellular components into the surrounding tissues. Researchers' consideration of these components, which result directly from tissue invasion and degradation, is well merited in studying the angiogenic response within an avascular tissue. Using samples of human tumor cell lines to initiate an angiogenic response from limbal vessels surrounding the cornea revealed crucial observations concerning tumor-associated angiogenic mechanisms that are still being elaborated on today. Another of these observations – the preangiogenic migration of macrophages and monocytes immediately ahead of proliferating capillaries – is quite noteworthy because of its prevalence in inflammation, bacterial infections, and wound repair(12,29).

More specifically, many of the growth factors and secretion products that macrophages produce are able to bind to extracellular matrix (ECM) constituents (45). These secretion products are often associated with angiogenesis and inflammation via their interaction with lymphocytes, fibroblasts, and various epithelial cell forms. Clarification of the interactions between macrophage products and ECM components or analogs thereof might lead to the clinical utilization of stabilizing ECM components.

For example, low molecular weight heparan sulfate proteoglycan polymers, heparin (a highly sulfated structural analog to heparan sulfate proteoglycan), or heparin plus hydrocortisone are each known to markedly reduce the microvessel formation in the cauterized cornea (30). Heparin specifically inhibits platelet aggregation, hence disabling the coagulation of blood in the lung and is rapidly taken up by lung cellular components (50,51). Heparin also binds to macrophage inflammatory proteins 1 alpha, 1 beta, and 2, which mediate inflammation or lung injury induced by interleukin-1 (IL-1), a proinflammatory cytokine (cell-produced activator) (52,53). Assessment of these clinical applications of heparin and of the binding capacity of heparin to macrophage growth factors suggests that heparin competitively inhibits these growth factors' binding to heparan sulfate proteoglycan, subsequently inhibiting macrophages' regulation of blood coagulation.

The importance of heparan sulfate proteoglycan (HSP) in maintenance of the structural integrity of the ECM is evident by its prominent organization in the basal lamina secreted by cells as part of their anchoring ECM membrane. Jerdan et al. showed that HSP, as well as laminin and collagen IV, are associated with actively invading capillaries in angiogenesis (54). Vlodovasky et al. provided evidence that suggests the

mechanism of lung metastasis to be directly related to the production of heparanase, which degrades HSP, by tumor cells (45). Invading capillary sprouts with migrating macrophages might specifically use growth factors to disrupt or depolymerize the HSP thereby facilitating angiogenesis. Angiogenic growth factors, particularly those inactivated and stabilized by being bound up in ECM components such as HSP polymers could be released hence producing an angiogenic response leading to tumor growth.

Kopf-Maier et al. observed that HSP, laminin, and collagen IV of the basal laminae of human lung carcinomas that are transplanted into mice, aggregates into patches and are discontinuous after first being degraded by invading macrophages (55). Regarding a lung tumor's more extensive invasive activities, proliferation, and the recognition thereof as native tissue in comparison to macrophage activity, one would consider the disrupting activities of macrophages to be tumor-promoting. Quite possibly, the ability of macrophages to successfully mediate angiogenesis depends on their ability to recruit other cells, thereby employing positive feedback mechanisms to promote or inhibit angiogenesis when needed during wound repair.

The roles of ECM collagen, cellular-adhesion molecules (e.g. fibronectin and laminin), and the integrins are especially important in migration and interaction of macrophages, lung tumor cells, and vascular endothelial cells. The presence of collagen IV antibodies in sera of lung-cancer patients has recently been identified as being an important prognostic factor due to its differential appearance in normal versus tumorous lung-tissue basement membranes (thicker variants of basal laminae) (56). Fernandez-Madrid et al. noted that these antibodies contribute to differences in the length of disease-free survival and may indicate the histological cell type of the lung tumor. Moreover,

Polette et al. was able to identify that the distribution of various collagen chains in the basement membrane is directly related to the histological types of bronchogenic carcinoma (57). Though increased expression of collagen IV tends to be localized within the walls of blood vessels in a lung tumor's vicinity, the increased presence of collagen IV seems to suggest that it plays a role in the reinforcement of the basic structural integrity of basal laminae of blood vasculature and that it is utilized mostly for vascular endothelial or lung epithelial cell anchorage. ECM-macrophage interactions are quite often associated with collagen I, which is usually used in migration assays for such cells (58).

Fibronectin, tenascin, laminin, and the integrins are quite renowned for their roles in extracellular and intercellular adhesion and cell migration. With respect to transmembraneous integrins, most lung-cancer cell lines will bind to the collagen I, laminin, and fibronectin, as do alveolar (lung) macrophages (58). Fibronectin is more associated with vascular endothelial cell surfaces and among infiltrating leukocytes around the vasculature than in noninflamed lung tissues (59,60). In non-tumor-derived inflammation of the lung, alveolar macrophages are able to aggregate across increased amounts of fibronectin on these vascular endothelial cells (61). Interestingly, macrophages express various alpha 5 beta 1 integrins that are localized on their pseudopodia and microvilli (62). This expression suggests a means by which they migrate across fibronectin. Following induction of inflammation of the lung, these macrophage integrins are found to have an especially high affinity to bind to fibronectin (63). More specifically, alpha 5 beta 1 is known to be rather specific for fibronectin and is required for the formation of an anti-tumorigenic fibronectin matrix (64,65). The

expression of this integrin by macrophages may be useful in constructing an enhanced anti-tumoral therapy against forms of lung cancer that express a fibronectin matrix.

In some cases, tenascin competitively inhibits the binding of cellular alpha V integrins of lung epithelial cells to fibronectin. For example, it has been shown that alpha V beta 6 integrins can bind both to tenascin and fibronectin (59). This results from tenascin having several protein sequences that are equivalent to those of fibronectin (66). In lung tumors, tenascin tends to penetrate the tumor mass whereas the presence of fibronectin is located mostly at the periphery of the tumor (60). Hence, the proliferation of lung tumors might directly depend on their ability to break down significant amounts of ECM components that macrophages and other immune cells depend upon to migrate or to induce ECM production via integrins. It might be interesting for one to construct an experiment using macrophages that can be engineered or stimulated to express competitive integrins, the alpha V beta 6 integrin for example, to determine whether they are able to reduce the growth of a lung tumor *in vivo*.

Quite possibly, vascular endothelial or cancerous cells, which are not associated with as much fibronectin as are those in inflamed lung tissues, are also not as well stabilized and would migrate and proliferate more. Pasqualini et al. showed that intraperitoneally injected fibronectin significantly inhibits lung colonization from the intravenously injected tumor cells (experimental metastasis) (67). They also demonstrated that this polymeric form of fibronectin prevents tumor formation if administered to carcinomas prior to their inoculation into mice. Though it has already been observed that fibronectin tends to encase a lung tumor mass (62), the clinicians use of specific ECM components intravenously (e.g. endostatin) or in a digestible form (e.g.

heparin plus corticosteroids) to inhibit the invasive activity of a lung tumor might prove to be successful. However, there are still other ECM components and macrophage products to consider because of their direct or indirect association with the ECM and the various cells therein.

Interferon-gamma-Activated Macrophages: Extracellular Matrix Interactions

Macrophages are capable of secreting a vast array of growth factors and, as noted previously, are quite versatile in migrating and in recruiting other cells from blood vessels and the ECM. By understanding the exact mechanisms correlated to certain pathological conditions, such as inflammation, fibrosis, angiogenesis, and metaplasia (alteration of a tissue's function due to misuse), researchers may identify several points of control for use by clinicians during clinical trials. So far, the role of macrophages' ECM interactions is deemed fairly important in terms of lung-disease pathology. Many of these ECM interactions are made possible via interactions with other cells such as fibroblasts.

The expression of alpha V integrins by the macrophages can be strongly affected by the presence of interferon-gamma (IFN-γ), one of the most potent stimulators of macrophage growth-factor production (68-71). Kohn et al. demonstrated that the presence of inflammation in a tissue might ultimately result from IFN-γ's down-regulation of alpha V (fibronectin receptor) integrin expression on peripheral blood monocytes and monocyte-derived macrophages (65). They indicated that these monocytes become detached from vascular endothelial cells and basement membranes quite possibly during inflammation in the presence of IFN-γ. These monocytes as well as

infiltrating leukocytes, which may, in turn, stimulate monocytes to differentiate into macrophages, become deposited at sites of inflammation (61). The ability of monocyte-derived macrophages to resist changes in fibronectin receptor expression when stimulated by IFN-γ is a particularly interesting observation because IFN-γ is also known to augment macrophage growth-factor production (65). If IFN-γ were to cause macrophages to produce growth factors and simultaneously to become detached from fibronectin, then energy expended by leukocytes to induce macrophage production of growth factors via IFN-γ would be relatively wasted. The migration of IFN-γ-activated macrophages towards sites of inflammation could possibly be directly dependent on expression of integrins of its pseudopodia that are specific for the ECM (e.g. fibronectin) of the inflamed lung tissue.

The interaction of a vast number of growth factors produced by or affecting macrophages has a prominent importance relating to specific integrins and their ECM ligands. Freidlander et al. reported that basic fibroblast growth factor (bFGF) or tumor necrosis factor-alpha (TNF-alpha) depend upon the vitronectin receptor, alpha V beta 3 integrin, to elicit an angiogenic response in the corneal angiogenesis assay, whereas angiogenesis induced via vascular endothelial growth factor (VEGF) or transforming growth factor-alpha (TGF-alpha) depends on the alpha V beta 5 integrin (68). TNF-alpha and TGF-alpha are understood to be produced by IFN-γ-activated macrophages. VEGF is known to be expressed by macrophages, pituitary cells, and lung epithelial cells. Involvement of specific alpha V integrins expressed by macrophages and epithelial cells in angiogenesis mediated via growth factors indicates the relative importance of these integrins and their respective ECM ligands in lung tumor development.

Lymphocytes (immunoactive leukocytes) and/or the presence of several growth factors bound in the ECM may also activate monocytes/macrophages. The cytotoxic lymphocyte maturation factor interleukin 12 (IL-12), which is produced by macrophage cells, is able to inhibit a wide variety of tumors *in vivo*, is anti-angiogenic in the mouse corneal assay, and is found to mediate such a response *in vivo* through IFN-γ (72-74). Normally macrophages are able to stimulate lymphocytes to produce IFN-γ by secreting IL-12, which is, in turn, significantly inhibited or impaired by transforming growth factor beta 2 (TGF-beta2), another macrophage product (75). Lymphokines, lymphocyte-secreted activators, such as IFN-γ and interleukin-2 (a mediator of IFN-γ) are concomitantly produced by lymphocytes in the presence of lung inflammation, pathological conditions, or simply in culture without external induction (76-79). In an immunotherapeutic trial with lung-cancer patients, Hanagiri et al. were able to boost the cytotoxicity of the patients' resected regional lymph node lymphocytes via culturing them with IL-12 and either low-dosage of IL-2 or high-dosage IL-2 with hydrocortisone (74). Reasonably enough, these lymphocytes produced IFN-γ and TNF-alpha in addition to its cytotoxic activity. With respect to the characteristic involvement of lymphocytes in the immune response against invasive microbial infections, the recruitment of lymphocytes by macrophages or vice versa might be suggestive of how macrophages react to invasive lung tumors.

The importance of lymphocytes becomes quite clear when one considers their role in inducing alveolar macrophages to mediate angiogenesis. Though alveolar macrophages produce factors that suppress lymphocyte proliferation, Upham et al. demonstrated that they do not inhibit activation of lymphocytes or lymphocytes'

production of certain cytokines (76). It is considerably reasonable to suggest that macrophages using IL-12 to activate lymphocytes' production of IFN-γ do not simultaneously cause them to detach, thereby defeating the purpose of activating their secretion of IFN-γ (80). It is also reasonable to suggest that certain macrophage activities are more necessary at specific stages of the immune response, thus requiring macrophages to inhibit further T-lymphocyte proliferation that might interfere or impede their activity. If macrophages are unable to migrate into a tumor mass due to lack of sufficient lung epithelial cell expression of ECM components, this might allow for tumor proliferation resulting from insufficient immunoregulation from macrophages. Though not always necessary (81), sufficient immunoregulation from macrophages might allow for the IFN-γ-production by lymphocytes, thus resulting in an augmented anti-tumoral activity from macrophages and lymphocytes.

Overall, IFN-γ is a relatively potent stimulator of the tumoricidal activity of macrophages (82). IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF), a lymphokine that induces the differentiation of monocytes into macrophages, are both used to activate macrophages that are being used in the initial phases of clinical trials to halt metastatic cancer. The successful use of these activated macrophages in clinical trials would then indicate that their abilities, possibly including the angiogenic mediation, in normal and neoplastic tissues should be considered rather significant.

Even in Gimbrone et al.'s first report of a novel angiogenic assay, the prolonged effect of the infiltrating blood vessels in some cases (epitheliomas, carcinomas) is to degrade the tumoral mass after it has proliferated and inflammation has occurred (12). In the other tumor cell lines they studied, some tumor masses are, for the most part,

unabated by the proliferating vessels. Thus, their results indicate that a positive feedback mechanism is initiated by the tumor, that there is a lack of sufficient mediation from macrophages that regulate angiogenesis and fibrotic synthesis (scar development), or that there is simply a deficiency in the number of "host" cells required to control the invasive, exponential growth.

Macrophage activity induced by activators other than IFN-γ (e.g. IL-4/corticosteroids) might also be vulnerable to tumor invasiveness or be insufficient to a degree that results in net growth of the tumor (83,84). A study by Kodelja et al. indicates that these "alternately-activated" macrophages are typical of chronic inflammation and wound repair (83). In fact, they induce about three times as much vascular endothelial cell proliferation as do IFN-γ-activated macrophages, which are more typical of early inflammation and high turnover granulomas (mass of granulation tissue produced in response to chronic infection, inflammation, or to a foreign body). If alternately-activated-macrophage growth factors stimulate vascular endothelial cells toward excess angiogenic activity in the absence of IFN-γ-activated macrophage mediation, a tumor might experience a net growth or metastasize, leading to the negative prognosis associated with tumor angiogenesis and metastasis.

When considering that activated macrophages are capable of paracrine and autocrine mediation (83,84), one can deduce that macrophages are well suited to mediate angiogenesis under conditions enabling them to recruit IFN-γ-secreting leukocytes (e.g. lymphocytes). Subsequently, the combination of activated macrophages and lymphocytes is able to mediate the vascular endothelial cell proliferation and migration depending directly on the presence of specific ECM constituents such as collagen I and

fibronectin (85). Angiogenesis in a tissue may differ from those in its cancerous counterpart, but, when considering the exact composition of the ECM within and around the vasculature, researchers may still be able to determine the exact angiogenic control points with respect to the basic ECM composition in a specific type of tumor.

Angiogenic Roles of Activated-Macrophage Growth Factors, Vascular Endothelial Cells, and Lung Fibroblasts

Angiogenic mediation by activated macrophages largely depends upon the production of growth factors by lymphocytes, monocytes, and other macrophages as well as the production of an ECM by fibroblasts and vascular endothelial cells (VECs). Activated macrophages produce a wide range of angiogenic factors *in vivo* that regulate several angiogenic pathways requiring the presence of these other resident cells. IFN-γ stimulates macrophages to produce several angiogenic factors and mediators, such as platelet-derived growth factors-A and –B (PDGF-A, -B), transforming growth factors-alpha and -beta 1 (TGF-alpha, -beta 1), insulin-like growth factor-1 (IGF-1), tumor necrosis factor-alpha (TNF-alpha), and hepatocyte growth factor (HGF/SF) (83). In certain cases, many of these growth factors abrogate further angiogenesis via their induction of other cells to produce anti-angiogenic factors.

Several regulatory growth factors that IFN-γ-activated or alternately-activated macrophages produce are, by themselves, potently angiogenic, even though they are still able to induce anti-angiogenic activity *in vivo*. Kodelja et al. noted that alternately-activated macrophages produce IGF-1, TGF-beta 1, PDGF-B, and HGF/SF but not TNF-alpha as compared to IFN-γ-activated-macrophage growth-factor production (83). Furthermore, a study by Cornelius et al. showed that IFN-γ inhibits the TNF-alpha-

induced production of collagenase, a matrix metalloproteinase (MMP) enzyme that catalyzes the degradation of collagen and is highly expressed in migrating vascular endothelial cells (VECs) (86). Kodelja et al. suggested that these alternately-activated macrophages might produce a three-fold greater proliferation response by VECs *in vitro* than IFN-γ-activated macrophages because IFN-γ is either directly or indirectly inhibiting growth factor-induced (e.g. TNF-alpha-induced) angiogenic activity (83). In particular, several other studies confirm that the presence of IFN-γ is strongly associated with the inability of VECs to adhere to collagen, fibronectin, or other extracellular matrix surfaces (69,72,87). Therefore, VEC migration in tissues must directly depend on the presence of collagen, the VEC expression of collagenase to migrate within the tissue matrix, and the angiogenic activities of macrophages and lymphocytes (i.e. growth-factor production). Whether the production of a specific growth factor ultimately depends on which extracellular matrix molecules are present or not remains in question.

Though VEC migration is indirectly stimulated by IFN-γ's induction of macrophage growth-factor production, these studies also demonstrate IFN-γ's ability to directly inhibit migration of cultured VECs. If one considers that IFN-γ concomitantly up-regulates VECs' production of tissue inhibitors of MMPs (TIMPs) as well as inhibits their production of collagenase (TNF-alpha-induced), VECs must possess the abilities to inhibit and/or promote their own movement with respect to the presence of TNF-alpha and IFN-γ. As Cornelius et al. demonstrated, IFN-γ not only inhibits TNF-alpha-induced or phorbol ester-induced collagenase production by VECs but upregulates their expression of TIMP-1, an inhibitor of MMPs, also (86). By inducing lymphocytes to produce IFN-γ via macrophage secretion of IL-12, macrophages might be able to induce

a strictly mediated angiogenic response with respect to which macrophage growth factors are produced. Accordingly, lymphocytes' IFN-γ-activation of macrophages in the presence of VECs might allow for this enhanced mediation of angiogenesis.

Besides being able to recruit lymphocytes and control VEC activity, activated macrophages possess the ability to recruit fibroblasts to reconstruct degraded matrices during inflammation beyond the effects of lymphocytes. IFN-γ secreted by lymphocytes has no effect on lung fibroblast replication in comparison to the stimulatory effects thereon by fibronectin (together with alveolar macrophage-derived growth factor) (88). Instead, as Bitterman et al. demonstrated, alveolar (lung) macrophages produce growth factors and interact with fibronectin in a way that stimulates fibroblasts to divide during interstitial lung disorders. The effect of IFN-γ on macrophages may indirectly stimulate lung fibroblast replication that in turn results in pulmonary fibrosis (88,89) – a thickening and stiffening of alveolar sacks in the lungs.

For comparison, a study by Norioka et al. showed that IFN-γ enhances the anti-migratory and anti-proliferative effects of interleukin-1 (IL-1), a proinflammatory macrophage product, on VECs by interference with their adhesion to a collagen matrix (87). This enhancement of inflammatory activity by IFN-γ as well as its overstimulation of macrophages to induce fibrotic activity may indicate that the lymphocyte activity should be considered more thoroughly or that macrophages' mediation of angiogenesis and fibrogenic activity is deficient. Simple examination of cultured macrophages, lymphocytes, and fibroblasts may reveal vital information on exactly what mechanisms of inflammation, angiogenesis, and fibrogenesis are being utilized to repair degraded tissues (90,91).

IL-1 is significantly associated with angiogenesis and inflammation in several pathological conditions. However, as Peuringer et al. noted, IL-1 and the macrophage product it induces, prostaglandin E_2 (PGE_2), which inhibits fibroblast replication, are poor clinical markers of alveolar macrophage activity during fibrosis when taken from bronchoalveolar lavage fluid samples (89). These researchers also noted that PGE_2 is produced by alveolar macrophages when taken from patients with such chronic interstitial disorders as fibrosis or sarcoidosis and then placed in culture. For comparison, a study by Schwartz et al. showed that PGE_2 is also produced in alveolar macrophages from patients with restrictive lung functions resulting from asbestos exposure (92). This non-metastatic, antigenic activity in the lungs may be the sole cause for PGE_2, as seen in the bronchoalveolar lavage fluid of lung cancer patients. The clinical marker for such fibrotic activity and inflammation in lung cancer patients may actually turn out to be their alveolar-macrophages' production of PGE_2 in culture (93-97).

Interestingly enough, the bronchoalveolar lavage fluid from asbestos patients shows a significantly higher concentration of fibronectin than that of normal patients (92). This increased amount of loose fibronectin may result from the relative inflexibility of asbestos fibers and their disruption of the extracellular matrix during lung movement. As mentioned before, polymeric fibronectin is being used to halt metastatic cancers (67), though such treatment in the case of lung-cancer patients with fibronectin in their bronchoalveolar lavage may turn out to be useless because of the cancers' apparent degradation of fibronectin. In either case, the disruption of fibronectin polymers that stabilize the lung might certainly impair activated macrophage migration and activity. If macrophages were engineered to express other integrins that bind to other parts of the

ECM not found in the bronchoalveolar lavage fluid, then this may allow for anti-tumoral activity of the macrophages to continue. Additionally, the enhancement of fibroblasts or lymphocytes activities may aid the macrophages in eliciting an appropriate anti-tumoral response in lung cancer patients.

Angiogenic Roles of Hepatocyte Growth Factor/Scatter Factor and Thrombospondin-1 in Bronchogenic Carcinoma

Activation of macrophages may result in the their production of a numerous amount of factors *in vitro* but may not necessarily account for all that occurs *in vivo*. Determining whether hepatocyte growth factor/scatter factor (HGF/SF) by alveolar macrophages and vascular endothelial cells is secreted in the lungs *in vivo* may be partly achieved by determining the types of ECM constituents and then assessing the competitive binding activity of HGF/SF for them (98). Already, collagen I and IV, fibrinogen, tenascin, laminin, and heparan sulfate proteoglycan are known to be vital components in maintaining the structural integrity and architecture of the lungs. Interactions between these ECM constituents as well as between them and HGF/SF may help determine the exact angiogenic nature of HGF/SF in normal and cancerous human lungs.

The evidence supporting HGF/SF's clinical role in bronchogenic carcinoma has begun to be recognized within the past 10 years. The most significant correlations tend to be with the recurrence rate or metastatic behavior of the cancer, both of which indicate a relatively poor prognosis. Olivero et al. showed that elevated levels of HGF/SF and its receptor are strongly correlated with poor prognosis for stage I NSCLC patients and even more so with the later stages (II, IIIa) of NSCLC (99). Another clinical study by

Takigawa et al. revealed that HGF/SF serum levels are significantly higher in patients with small cell lung cancer (SCLC) specimens, moreso in those with bacterial pneumonia, and significantly lower in those with benign lung tumors (100). In this study of patients with invasive or inflammatory lung diseases, the presence of HGF/SF is directly correlated with the degree of invasiveness of conditions such as bacterial pneumonia, SCLC, and NSCLC. The evidence compiled here suggests that the additional presence and/or increased levels of angiogenesis factors in lung tumors are induced by the presence of anti-angiogenic, inflammatory agents produced either by the invasive "force" and/or by macrophage/monocyte mediation as a natural reaction to such invasion.

However, the strong correlation between high HGF/SF levels and the invasive nature of lung carcinomas should not be overlooked. As indicated previously, such invasiveness also correlated with higher levels of fibrinogen in the bronchoalveolar lavage rather than IL-1 or PGE_2 (92). Sakai et al. noted that significantly higher levels of HGF/SF are detectable in the bronchoalveolar lavage fluid during pulmonary fibrosis and inflammation (101). These findings suggest that concentrations of both growth factors and ECM components released into the extracellular body fluids are significantly related to an invasive pathological origin and might be used to identify the exact nature of a lung carcinoma. Furthermore, the concentrations determined from culturing resident cell types may allow clinicians to determine which anti-tumoral therapy is most effective.

Both alveolar macrophages and lung endothelial cells can secrete HGF/SF in the lung during wound-healing, organ regeneration, and/or carcinogenesis. HGF/SF is known to be able to induce the proliferation and migration of lung epithelial (alveolar type II)

cells while inducing no such reaction from lung fibroblasts (102). Nakamura et al. found that several human lung-cancer cell lines produce IL-1, basic fibroblast growth factor (bFGF), and PDGF, all of which strongly stimulate the production of HGF/SF in human skin fibroblasts (98,103). Additionally, Olivero et al. demonstrated that both normal and cancerous human lung epithelial tissues express the receptor for HGF/SF, whereas lung fibroblasts produce HGF/SF (99). More specifically, Sakai et al. considered that HGF/SF's role in pulmonary fibrosis may be through a paracrine mechanism and could possibly be involved in the healing of the inflammatory damage in the lung's ECM (101). These correlations of HGF/SF with ECM-related pathogenesis may further elucidate its angiogenic role in the mechanisms of lung tumor pathogenesis (104).

Since HGF/SF is known to induce tumor cell motility and invasion as well as angiogenesis *in vivo*, its mechanism of action is extremely noteworthy in terms of interactions with the components of the extracellular matrix components and other growth factors. For example, HGF/SF has binding capacities for fibronectin and heparan sulfate proteoglycan in breast cancer cell lines and is dependent on the presence of collagen IV, laminin, or fibronectin on culture surfaces to stimulate the migration of glioma cells (105,106). As noted before, collagen IV, laminin, and heparan sulfate proteoglycan are all associated with VEC movement in angiogenesis (54). Assessment of HGF/SF's role in ECM-related, angiogenic activities shows that it binds strongly either to dermatan sulfate or heparan sulfate proteoglycans, the latter of which is notably important in the proliferation of VECs in angiogenesis (107). Heparin, an angiogenic mast cell product, is also found to competitively inhibit the binding of such proteoglycans and their analogs to the HGF/SF in human breast carcinomas. Nonetheless, heparin is

able to potently inhibit angiogenesis when administered with cortisone, as do its fragments in the presence of certain corticosteroids (2,30). In the case of human gliomas, heparin enhances the anti-neoplastic activity of cortisone, which is comparable to its anti-angiogenic activity in the rabbit corneal assay. Quite possibly, the competitive binding activities of heparin and its ECM analogs thereof for growth factors (e.g. HGF/SF) are directly responsible for the potent anti-angiogenic properties of heparin in *in vivo* studies of lung cancer.

Evidently, HGF/SF has the highest affinity to bind to a large, anti-angiogenic, anti-adhesive, matrix glycoprotein, thrombospondin-1 (TSP-1), instead of heparan sulfate proteoglycan, fibrinogen, or seven other ECM components from breast-cancers' stromae (106). Lamszus et al. suggested that the high affinity binding of HGF/SF to TSP-1 modulates an angiogenic response in the presence of invasive carcinomas. Jin et al. found that high IL-1beta content in invasive breast carcinomas is significantly associated with higher contents of both HGF/SF and TSP-1 (108). Considering that HGF/SF and TSP-1 have a strong affinity to bind and that IL-1beta is more directly associated with inflammation, one may infer that high HGF/SF-TSP-1 content is also correlated with the invasiveness and high angiogenic activity of such carcinomas.

Wound repair relies heavily on persistent mediation by TSP-1-producing macrophages. TSP-1 is normally secreted by platelets, fibroblasts, vascular endothelial cells, and especially by macrophages during early wound repair (109-112). As reported by DiPietro et al., after inhibiting the quantity of macrophages producing TSP-1, wound repair is substantially delayed (111). Further studies by DiPietro et al. strongly indicated that the macrophages regulate angiogenesis during wound repair by increasing TSP-1

production, at first, then by steadily decreasing it after day 1 until TSP-1 is completely undetectable on day 10 (113). Since these high concentrations of TSP-1 are also associated with high angiogenic activity in invasive, angioproliferative tumors, it can be deduced that a net angiogenic effect would be observed in the presence of TSP-1 or other matrix components for as long as HGF/SF or other such angiogenic growth factors are produced in excess of TSP-1.

TSP-1 and HGF/SF have an interestingly vital role in the architectural construction and the maintenance of such in the lungs, whether with inflammation, lung cancer, or simply developmental processes. A study by Tucker et al., pointed out that TSP-1 is consistently expressed in the development of nonvascular tissues and in the tips of developing, avian lung bronchioles (114). Kuhn et al. also confirmed that TSP-1 is consistently expressed in organizing pneumonia, not too often in fibrosis, and plays a major role in the anchoring the repaired epithelial layers during wound healing. The key activity of TSP-1 in such processes may be a result of its ability to competitively bind to growth factors such as HGF/SF. Interestingly enough, human collagen XVIII from which endostatin is derived has several collagenous and noncollagenous homologous portions and, in particular, contains a noncollagenous thrombospondin homologous section (116). TSP-1's anti-angiogenic activity may be similar to endostatin's, though TSP-1 is naturally utilized in wound-repair processes and can be inhibited from binding to other growth factors via heparin's competitive binding activities (117,118). TSP-1's anti-angiogenic activity may be controlled via activated macrophage secretion of several other growth factors besides HGF/SF, such as platelet-derived growth factor and transforming growth factor-beta as is determined in the next section.

Angiogenic Roles of Transforming Growth Factor-beta, Platelet Derived Growth Factor, and Thrombsondin-1

Evidence implicating transforming growth factor-beta's (TGF-beta's) importance in lung cancer also indicates the importance of other growth factors and ECM components such as platelet-derived growth factor (PDGF) and TSP-1 in bronchogenic carcinoma. Several studies of bronchogenic carcinoma have recently correlated TGF-beta plasma levels with lung inflammation and the lack or presence of TGF-beta receptors with various types of lung cancer and other pulmonary conditions (119-123). To better understand the angiogenic roles and interactions between TGF-beta and PDGF, one must understand more about the activity of TSP-1 in with cellular element of tissues.

Castle et al. determined that transformed fibroblasts are not able to form tumorous growths because of the overexpression of TSP-1 and TSP-1's inhibition of microvessel formation *in vivo*; but even when the transformed fibroblasts overexpress TSP-1, they are still characterized by serum and anchorage independent proliferation in vitro (112). A study by Hsu et al. showed that glioblastoma multiforme cell lines, which are intensely angiogenic and lack normal expression of human chromosome 10, switch from their angiogenic phenotype to an anti-angiogenic one when a wild-type chromosome 10 is returned to them and increases their ability to express TSP-1 (124). Hence, the absence of normal TSP-1 expression may result in the highly angiogenic phenotype of malignant cancers, whereas TSP-1's overexpression may potentiate an anti-angiogenic response *in vivo*.

Other macrophage-derived growth factors that can bind to TSP-1 such as platelet-derived growth factor-BB (PDGF-BB) and transforming growth factor-beta 1 (TGF-beta

1) have prominent regulatory roles in the angiogenic process. PDGF-BB specifically binds to TSP-1, thereby non-competitively inhibiting and reducing by approximately five-fold the binding of PDGF-BB to its receptor on vascular smooth muscle cells (VSMCs) (125). In addition to growth and migration of vascular endothelial cells, such activities are also characteristic of VSMCs during wound repair involving larger blood vessels (110,126,127). Whereas PDGF-BB normally mediates this growth and migration of VSMCs by inducing the production of several ECM proteins including TSP-1, both TSP-1 and PDGF-AB stimulate the growth and migration of mesangial (glomerular) simple squamous epithelial cells and are produced by them as well (126). This autocrine mechanism involving PDGF and TSP-1 is also present in VECs and is further regulated by TGF-beta 1, a strongly anti-angiogenic factor as demonstrated in three-dimensional collagen gels (128).

TGF-beta 1 has an extensive role in angiogenesis and cell proliferation and is known to affect all vascular cells, VSMCs and VECs in particular, and a wide variety of other normal and neoplastic cell types throughout the body. Janat et al. demonstrated that TGF-beta 1 prolongs an increase of TSP-1 and collagen IV production by VSMCs as compared to PDGF-BB, which causes a stronger, more acute yet temporary increase in TSP-1 that is further enhanced by the addition of TGF-beta 1 (127,129). Considering that TSP-1 and TGF-beta 1 are normally bound as platelet alpha granules, TSP-1 activates the two forms of "latent" TGF-beta by binding to it as Schultz-Cherry et al. demonstrated (130). Their results show that activated TGF-beta-TSP-1 complexes increase the incidence of NRK-49F cells to form increased numbers of colonies. The activity of such complexes may explain the ability of TGF-beta to promote the formation of tube-like

structures by VECs in three-dimensional collagen gels. Jennings et al. demonstrated that PDGF-AA, -BB, -AB, alpha PDGF and/or beta PDGF subunits are all means through which TGF-beta stimulated DNA synthesis in human glioblastoma multiforme cell lines (HD-GM) (131). The aforementioned findings verify that PDGF can serve as the principal mediator in TGF-beta's autocrine induction of further TGF-beta, PDGF, and ECM components' production such as TSP-1, collagen type IV, and fibronectin (132); in the absence of TGF-beta's regulatory actions, PDGF may cause a strong, acute stimulation of ECM components' production.

However, Jennings et al. considered an interesting dilemma of TGF-beta exhibiting both an inhibitory effect on the HD-GM diploid cells and a mitogenic effect selectively in the aneuploid glioma cell lines (131). In concluding, they suggested that glioma cells exhibiting hyperdiploidy (aneuploidy) may proliferate by overproducing autocrine growth factors in the presence of TGF-beta instead of being inhibited by TGF-beta as in the diploid cell lines. The evidence supporting that conclusion also supports Castle et al.'s "notion that alterations in the net balance between inducers and inhibitors of angiogenesis are largely responsible for the sustained growth of tumors *in vivo* (112)." In the case of glioma cells, growth factors such as TGF-beta and PDGF might be produced in excess of ECM components being produced. For example, TGF-beta's induction of more PDGF than TGF-beta production might essentially overcome the ability of TGF-beta to regulate the ECM production and deposition induced by PDGF.

The overstimulation of PDGF or TGF-beta production followed by their induction of more ECM component production such as proteoglycans (133), fibronectin (134), TSP-1, which binds to PDGF, might suggest a mechanism by which fibroblasts (135) or

lung tumor cells (134,136) deposit and generate more ECM. This certainly seems to be the case with repaired lung epithelium, which has much TSP-1 incorporated into the epithelium's basal lamina (113,115,137). However, this mechanism might be regulated by alveolar-macrophages' induction of an excess of anti-angiogenic ECM-component production, thus resulting in net anti-angiogenic activity (138). Or, quite possibly, by the shear presence of previously established extracellular matrices, vascular basal laminae, or basement membranes, the components of which are known to be able to bind to multiple angiogenic factors, the growth factors then become all bound up, disabling their angiogenic, inflammatory wound-repair processes.

Reconsideration of the Relative Significance of Anti-angiogenic Therapy for Bronchogenic Carcinoma: Activated-Macrophages, Integrins, and the Extracellular Matrix

Overall, the consideration of lung cancer's prevention by clinicians and physicians is certainly the highest priority in mind. Physicians' recognition of symptoms commonly preceding the development of bronchogenic carcinoma could allow for preventive therapeutic measures to be taken. Though there may be no extant mass of tumor cells, anti-angiogenic therapy might be somewhat reasonable if it can be shown to aid the body's wound healing mechanisms. One particular study of 86 patients' biopsy specimens from hyperplastic, metaplastic, and potentially preneoplastic lesions of the bronchial mucosa revealed a trend of increasing microvessel count with increasing amount of inflammation, squamous cell metaplasia, and degrees of carcinoma *in situ* (139). Though bronchial metaplasia is difficult to distinguish from chronic bronchitis, cytology and radiography tests are the most efficient determiners of bronchogenic carcinoma (33). However, their predictive capacity is low and does not test positive until the cancer has steadily begun to advance.

The advantage of detecting a wide variety of growth factors and ECM constituents in body secretions and fluids is that one may be able to determine the exact

nature of the lung tumor and the specific type of respective anti-tumoral therapy required (56). Anti-angiogenesis, if developed in this manner may prove extremely beneficial for lung-cancer patients and could lead to using this therapy by itself. It may also be much less cytotoxic towards the rest of the body when the therapy is removed.

However, these possibilities remain to be extensively tested on a clinical basis. Even such promising new anti-angiogenic therapies such as those with endostatin may yield unexpected side effects that do not increase the length of survival in human subjects. The use of phage-peptide-chemotherapeutic treatments in response to specific integrins on the extracellular surfaces that are supposedly unique to a lung tumor may only yield a small increase in disease-free survival, if one considers that the cancers implanted or induced in these experimental mice are usually non-native tissue to the mice. Lung tumors that originate in humans are derived from the human's native lung tissue. Being able to differentiate between the mice's own tissues might indicate more hope than only differentiating between foreign and native tissues respective of the type of experimental animal.

Pasqualini et al. did achieve such results with their alphaVbeta3-ligand peptide sequences (140-142), but it will be some time before their research moves toward clinical trials as is happening with endostatin. With respect to the nature and specificity of ligand-integrin actions in the lung, the opportunities afforded by further experimental trials could still be serendipitously beneficial. By developing an anti-angiogenic therapy that utilizes the macrophages mediation abilities and takes into account the vital importance of the ECM constituents in angiogenesis and tumor proliferation *in vivo*, one may be sure that the resulting therapy could be non-cytotoxic, non-inducive of host drug

resistance, and ultimately could allow the host to develop its own anti-tumoral response via immune reactivity towards further incidences of lung cancer.

The body already reacts toward lung cancer as it does toward characteristic bacterial infections via the wound-healing processes (e.g. angiogenesis, inflammation, etc.) elaborated on previously. Further understanding of these analogous relationships to lung cancer as well as being able to recognize early symptoms of lung cancer by using such indicators as the relative concentrations of cultured alveolar macrophage, nodal lymphocyte, and lung epithelial cell excretions might lead to a drastic reduction in the incidence of bronchogenic carcinoma.

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